## Climate change impacts and microbial controls on northern peatland nitrogen cycling

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# Chapter 2

# Enzymology under global change: organic nitrogen turnover in alpine and sub-arctic soils

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## 2.1 Abstract

Understanding global change impacts on the globally important carbon storage in alpine, arctic, and sub-arctic soils requires knowledge of the mechanisms underlying the balance between plant primary productivity and decomposition. Given that nitrogen availability limits both processes, understanding the response of the soil nitrogen cycle to shifts in temperature and other global change factors is crucial for predicting the fate of cold biome carbon stores. Measurements of soil enzyme activities at different positions of the nitrogen cycling network are an important tool for this purpose. We review a selection of studies that provide data on potential enzyme activities across natural, seasonal and experimental gradients in cold biomes. Responses of enzyme activities to increased nitrogen availability and temperature are diverse and seasonal dynamics is often larger than differences due to experimental treatments, suggesting that enzyme expression is regulated by a combination of interacting factors reflecting both nutrient supply and demand. The extrapolation from potential enzyme activities to prediction of elemental nitrogen fluxes under field conditions remains challenging. Progress in molecular "omics" approaches may eventually facilitate deeper understanding of the links between soil microbial community structure and biogeochemical fluxes. In the meantime, accounting for effects of the soil spatial structure and *in situ* variations in pH and temperature, better mapping of the network of enzymatic processes and the identification of rate-limiting steps under different conditions should advance our ability to predict nitrogen fluxes.

## 2.2 Global change and the soil nitrogen cycle

It is predicted that global warming trends will continue to be most severe in arctic, subarctic and alpine environments (Christensen et al., 2007). Moreover, biological processes in these systems are often temperature limited (Shaver et al., 1992; Robinson, 2002). Due to the fact that soils are often close to the freezing point, non-linear effects of temperature around frequent freeze-thaw events can have large impacts (Deluca et al., 1992; Jefferies et al., 2010). Arctic and subarctic biomes constitute a globally significant carbon sink ( 30% of the global soil carbon pool) in the form of soil organic matter stored in peatlands and permafrost (Gorham, 1991). If changes to climate affect local biological processes in these ecosystems and consequently modify elemental fluxes, there is a potential for feedbacks to global biogeochemical cycles (Chapin et al., 2000; Aerts, 2006; Dorrepaal et al., 2009). Understanding the response of these important carbon sinks to higher temperatures is therefore crucial to predicting future biogeochemical cycles and climate.

Carbon storage in soil organic matter depends on the balance between carbon assimilation via primary production, and mineralization via decomposition. Although higher temperatures are expected to increase the rates of both of these processes in the short-term, their long term responses will depend on interactions with other potentially limiting factors such as soil moisture (Robinson, 2002; Aerts, 2006), oxygen availability (Freeman et al., 2001), nutrient availability (van Groenigen et al., 2006), and may also be affected by non-linear irreversible processes such as permafrost melting (Schuur et al., 2008). Nitrogen availability in particular is expected to constrain the response of primary production and decomposition to global change, given that both potentially are limited by nitrogen availability, particularly in colder biomes (Robinson, 2002; Mack et al., 2004). Due to the minimal inputs via atmospheric deposition and biological fixation, increased nitrogen supply has been found to relate closely to *in situ* decomposition rates of soil organic matter in pristine arctic and sub-arctic habitats (Robinson, 2002; Bragazza et al., 2006). Although plant species-specific factors can complicate models of litter decomposition (Aerts et al., 2006a), the close links between C- and N-cycling, underscore the importance of understanding the soil nitrogen cycle when attempting to predict the fate of cold-biome carbon stores in a warming climate.

Plants and microbes, constituting the main sinks for soil nitrogen, are both capable of assimilating low molecular weight forms of organic nitrogen such as amino acids (Clemmensen et al., 2008; McFarland et al., 2010), effectively short-circuiting the nitrogen cycle and leading to minimal levels of complete mineralization of organic

nitrogen to ammonia (Nannipieri & Eldor, 2009). For this reason, overall nitrogen cycling is regulated by the process of depolymerization, defined as the enzymatic release of labile low molecular weight nitrogenous molecules (amino acids, amino sugars, peptides) from the complex forms that make up soil organic matter (Schulten & Schnitzer, 1998; Schimel & Bennett, 2004).

In contrast to constructed N budgets, direct measurements of the enzymatic processes that contribute to the total flux provide information about the drivers and modifiers of individual steps in a complex network of interacting fluxes - information that is useful for gaining a mechanistic and ultimately predictive understanding of soil nitrogen cycling under global change. A range of standardized soil enzyme assays for a large number of reaction substrates has been developed (Allison et al., 2007; Wallenstein & Weintraub, 2008), which allow the comparison of soil enzymatic potentials across ecosystems (Sinsabaugh et al., 2008). In particular the use of substrates containing a fluorogenic component have increased sensitivity and lowered detection limits relative to colorimetry-based methods (Freeman et al., 1995). Figure 2-1 shows a selection of enzyme assays available for measuring different steps in the pathway from polymeric soil organic nitrogen to mineralization. Unfortunately, soil enzyme assays typically involve incubations of homogenized soil slurries at standardized temperature and buffer conditions with non-limiting amounts of substrate for a specified period of time. As a consequence, their relevance for estimating actual nitrogen fluxes may at times be questionable. The aim of this study was to review briefly the experiments that have investigated the effects of global change drivers on soil enzyme activities, with a focus on alpine and (sub-)arctic environments, so that we can subsequently discuss the challenges for translating enzyme assay data into predictions of soil nitrogen fluxes.

## 2.3 Global change effects on soil enzyme activities in temperature-limited systems

Understanding the direct effects of higher temperatures on soil nitrogen cycling requires knowledge of the kinetics and stoichiometry of decomposition processes (Davidson & Janssens, 2006; Sinsabaugh et al., 2009) and their responses to temperature. One aspect of this response is the temperature sensitivity of the reaction rate usually expressed as change in activity per 10 °C increase in temperature: $Q_{10}$. Although interpretation of $Q_{10}$ values can be influenced by the temperature range of their application (Davidson et al., 2006; Guicharnaud et al., 2010), they have proven useful

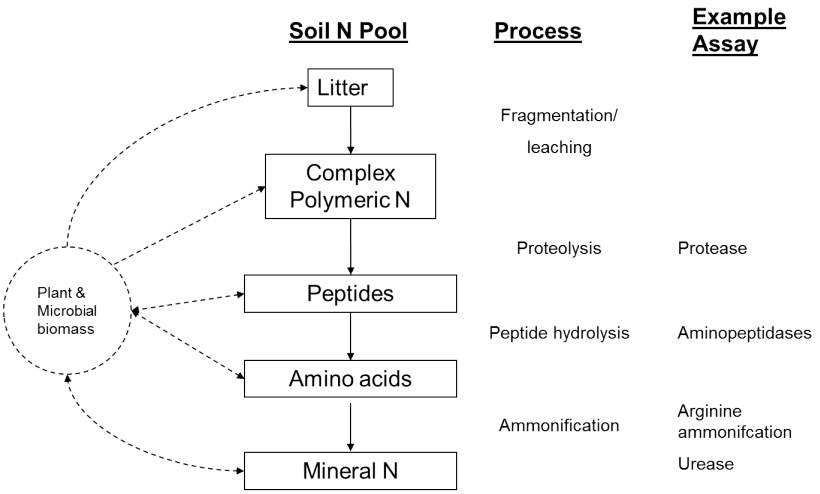


**Figure 2-1:** Simplified schematic diagram of the soil organic nitrogen cycle, including examples of assays available for quantification of potential rates of each process.

in the study of sensitivity of biological processes to changes in temperature. A study conducted in the Alaskan tundra suggests that carbon cycle enzymes are more temperature sensitive than nitrogen cycle enzymes (Wallenstein et al., 2009). This result is supported by experiments with alpine soils that additionally found that enzymes related to hydrolyzing labile compounds are more temperature sensitive than those associated with the breakdown of more stable soil organic matter (Koch et al., 2007). Given that many N-containing compounds are protected by carbon polymers, the responses of soil enzymes associated with both cycles need to be understood: If the differential response of C- and N-cycles to warming is consistent and long-lasting, then non-linear shifts in the stoichiometric balance of soil organic matter cycling, most likely towards enhanced nitrogen limitation, would be expected.

In addition to directly affecting enzyme activities, global warming may also increase nitrogen availability through other pathways. Studies that manipulate temperature in the field have the advantage of combining direct temperature effects on enzyme activities with a range of possible indirect effects mediated by e.g. longer term changes in vegetation composition and changes in nutrient supply via litter and grazing. These indirect effects may be even stronger than direct effects of global change drivers (temperature, $CO_2$, and precipitation) (Gutknecht et al., 2010), and it is therefore surprising that there is a relative lack of enzyme data from this type of study from arctic and alpine studies, despite the large number of climate manipulation experiments

currently underway (Walker et al., 2006). Several studies have, however, investigated the effects of increased mineral nitrogen inputs into soils on the dynamics of enzyme activities. Originally established to predict ecosystem impacts of anthropogenic increases in nitrogen deposition (Gruber & Galloway, 2008), these studies also provide important insights into the mechanisms of enzymatic regulation of soil nitrogen cycling. In this respect, it is still unclear whether measured soil enzyme activities reflect a response to nutrient deficiency or to availability — in other words, whether enzyme production by the microbial biomass is regulated by nutrient demand or supply. There is evidence available to support both possibilities (Weintraub & Schimel, 2005; Allison et al., 2007), which might be due to the complex enzyme regulation of the soil N-cycle. Chronic nitrogen fertilization of alpine tundra soils reduced leucine-aminopeptidase activities and increased urease activities while not affecting other N-cycle enzymes (Nemergut et al., 2008), while in an ombrotrophic bog in Scotland, N addition reduced potential activities of chitinase and cellulase, and altered the patterns of carbon substrate utilization (Currey et al., 2010). Similar complex responses have been found in other systems. Most strikingly, Enowashu et al. (2009) found that the activity of different types of peptidases in German spruce forest soils showed opposite responses to changes in mineral nitrogen input. Such disparate responses among enzymes with presumably similar functional roles point to a complex (and hard to predict) regulation of enzyme expression.

Further evidence on the effects of both temperature and nitrogen availability on enzyme activities is given by studies that measured seasonal dynamics, as seasonality can be understood as a driver that integrates simultaneous variance in temperature, moisture, plant and microbial nutrient demand and transport of nutrients into and out of the soil system. Studies that measured seasonal variation in potential enzyme activities have typically found large intra-annual differences (Weintraub & Schimel, 2005; Wallenstein et al., 2009; Currey et al., 2010), up to 10-fold (Currey et al., 2010), with such differences often being larger than the those detected in response to experimental manipulation of single factors (see above). In alpine, arctic, and subarctic environments, the highest enzyme activities seem to coincide with the period of microbial and nutrient turnover around the spring-thaw, during which frequent soil freeze-thaw cycles cause rapid, drastic shifts in the soil physico-chemical conditions (Jefferies et al., 2010) with consequences for the soil microbial community structure and associated functions (Yergeau & Kowalchuk, 2008).

This implies that global change effects on enzyme activities are: a) likely to involve interactive (i.e. non-additive) effects of changes to temperature and nutrient supply, and b) be mediated through changes to nutrient demand and supply both within the soil organic matter decomposing community, as well as in the associated vegetation (Chapman et al., 2006).

## 2.4 Extrapolating enzyme measurements to flux predictions: the missing link

Soil enzyme activity measurements are useful only insofar as they allow us to predict elemental fluxes and e.g. their responses to global change. Despite this, studies that explicitly link enzyme measurements to measurements of fluxes in the same experimental units are rare (e.g Carreiro et al., 2000; Sinsabaugh et al., 2002) and, to our knowledge, non-existent in (sub-) arctic and alpine environments. To illustrate what this link may look like, we present a synthesis of data from alpine (Koch et al., 2007) and arctic tundra (Weintraub & Schimel, 2003, 2005) in Figure 2-2. The measured activities of some enzymes were correlated with flux rates, i.e. nitrogen mineralization with xylosidase ($r^2$ =0.58, $P$ <0.05) and $\beta$-glucosidase ($r^2$=0.52, $P$ <0.05), and C-mineralization with protease activity ($r^2$=0.94, $P$ <0.05). However, not all enzyme activities seem to translate into elemental fluxes — as with N-mineralization vs. protease activity (Figure 2-2b) and C-mineralization vs. $\beta$-glucosidase and xylosidase in Figure 2-2a (i.e. none of these correlations were significant). This latter result is all the more surprising as $\beta$-glucosidase activity is traditionally considered a reliable proxy of soil microbial activity (Alef & Nannipieri, 1995).

Connecting potential enzyme activities, as reflected by enzyme assays, to actual enzyme activities *in situ* and, more crucially, realized elemental fluxes requires clarifying the influence of some important complicating factors. For instance, soil processes are spatially structured at multiple scales (Nannipieri & Eldor, 2009). Diffusion of enzymes and substrates within and between microhabitats (Ekschmitt et al., 2005) could potentially limit soil enzyme activities in ways that are not detectable by traditional soil enzyme assays that incorporate a homogenization step, particularly given the potentially important role of enzymes stabilized on soil particles (Wallenstein & Weintraub, 2008). Furthermore, enzymatic processes are sensitive to solution pH and temperature conditions. Standardized assay conditions, although useful for facilitating cross-system comparisons (Sinsabaugh et al., 2008), may lead to under- or overestimates of *in situ* potential rates (Freeman et al., 1995). Moreover, given that

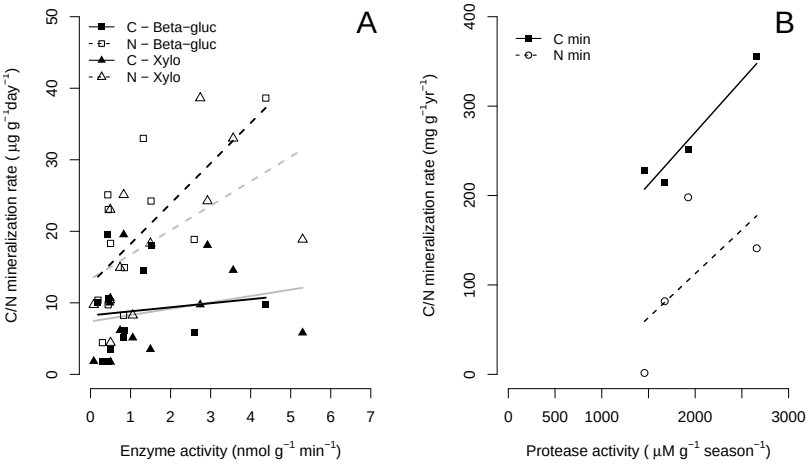


**Figure 2-2:** Relationships between measured enzyme activities and carbon and nitrogen mineralization rates as reported in (A) Koch et al.(2007) and (B) Weintraub and Schimel (2003,2005). Legend: filled symbols and solid lines = carbon mineralization, open symbols and dashed lines = nitrogen mineralization. For A, each datapoint represents a season x soil combination (summer wet fen soils excluded from linear trend line). Triangles and grey lines = xylosidase activity, squares and black lines = $\beta$-glucosidase activity. For B, each point represents four separate soil types, protease activity was calculated as cumulative potential activity by interpolation. See text for further statistical information.

temperature sensitivities have been shown to vary considerably between enzymes and across seasons (Koch et al., 2007; Wallenstein et al., 2009), extrapolations from assay data collected at a single temperature are potentially unreliable.

Probably the most serious factors complicating translation of potential enzyme activities to fluxes are the two (interrelated) problems of i) the unclear role of substrate availability, and ii) the complex, multi-step pathways of enzymatic intermediate reactions which ultimately lead to elemental fluxes. As mentioned above, it remains uncertain whether enzyme production is induced by nutrient deficiency (demand-driven) or by availability (supply-driven) (Weintraub & Schimel, 2005; Allison et al., 2007). This makes using potential activities as a guide for flux predictions problematic, as realized fluxes are a function of the actual substrate supply rate, as well as potential activity. If enzymes are produced in response to nutrient limitation, then assays that measure potential activities will have an inverse relationship to real fluxes in the field. Indeed, experimental approaches give support for an "economic" or

demand-driven model of enzyme regulation (Allison & Vitousek, 2005), and this has also been incorporated into a modelling framework (Sinsabaugh & Moorhead, 1997). Further investigation into this problem across a range of ecosystems will be an important step toward allowing us to make well-informed links between enzyme measurements and field-level nitrogen availability, particularly given the interaction between the latter and other global change factors. Similarly, the complex, multi-step pathway from polymeric organic nitrogen to mineral nitrogen (Figure 2-1) implies that measurements of the activity of a simple step in a complex network will not be sufficient for predicting total flux rates. Indeed, multiple soil nitrogen cycle networks are possible, each with different dominant nitrogen forms and different critical, rate-limiting steps (Schimel & Bennett, 2004; Nannipieri & Eldor, 2009). Given the multiple, indirect effects of global change drivers, it is conceivable that they can alter the relative importance of different N-cycle pathways, and therefore the critical, rate-limiting enzymatic processes determining elemental flux rates. All this argues for simultaneous measurements of enzymes in different positions of the nitrogen cycling network (Enowashu et al., 2009) (Figure 2-1), and further research into how global change alters the different pathways in such soil nitrogen flux networks (Schimel & Bennett, 2004).

Ultimately, the effects of global change on the soil nitrogen economy will always be filtered through the soil microbial community, the proximate driver of soil enzyme pool sizes and elemental fluxes (Allison et al., 2007; Wallenstein & Weintraub, 2008). Whether this filtering is best examined at the level of the composition of the soil microbial community (as detectable via metagenomics (Kowalchuk et al., 2007)), their transcriptional activity (metatranscriptomics (Urich et al., 2008)), their enzyme production (proteomics (Schulze, 2005)), or through soil enzyme assays remains an open question, although a combination of approaches will probably be the most fruitful approach. Molecular tools to predict enzyme activities have therefore been strongly advocated (Allison et al., 2007; Wallenstein & Weintraub, 2008). Indeed, in the long run, molecular methods are likely to offer considerable explanatory power in understanding soil community function once it has been established to which extent microbial communities are functional and kinetically redundant (Fierer et al., 2007), and once bioinformatic tools are sufficiently advanced to reliably predict functional characteristics of communities from environmental sequence data (Allison et al., 2007; Kowalchuk et al., 2007). Given the current state-of-the-art, we propose that a greater focus on the direct effects of environmental conditions and resource supply rates on elemental fluxes, and the enzyme processes that underlie them, will help to provide important information in the short term that should lead to considerable advances in the understanding of the global change effects on soil biogeochemical cycling.